VII - CLAIMING

What is claimed is:

1. The procedure for cloning human SMN gene based on the reverse transcription (RT) and the polymerase chain reaction (PCR) using the synthesized oligonucleotides (a) for RT, and (b) and (c) for PCR respectively, comprising:

- Isolation of SMN-mRNA.

- RT reaction using the synthesized oligonucleotide (a) with the following sequence: 5' TGGCAGACTTAC 3' (a).

- RT reaction conditions: 90°C for 2 minutes; 0°C for 1 minute; 25°C for 10 minutes; 42°C for 45 minutes.

- PCR reaction using the synthesized oligonucleotides (b) and (c) with the following sequences:
5' ATGGCGATGAGCAGCGG 3' (b).
5' TTAATTTAAGGAATGTGAGCAC 3' (c).

- PCR reaction conditions: Denaturation at 94°C for 1 minute; annealing at 55°C for 2 minutes; elongation at 72°C for 1 minute each cycle, for 35 cycles.

2. The procedure for the construction of expression plasmids using the pFastBac™ HTb and the pBlueBacHis2 A transfer vectors for the purpose of obtaining human SMN protein in insect cells, comprising:

2.1. Using the pFastBac™ HTb vector:

- Digesting the pFastBac™ HTb vector with BamHI and XhoI followed by dephosphorylation with calf intestinal alkaline phosphatase.

- Digesting the vector (1) pCR^R II/SMN-cDNA with BamHI and XhoI and isolating the resulting fragment containing the cDNA coding sequences of SMN protein, SMN- cDNA.

- Ligating the SMN-cDNA fragment to the pFastBac™ HTb vector and introducing the ligation product in INVαF' E. Coli strain.

- Screening for inserts based on the presence of white colonies, as a result of which the vector (2) pFastBac™ HTb/SMN-cDNA is selected.

- Introducing the vector (2) in DH10Bac™ E. Coli competent cells.

- Screening for recombinant bacmids in DH10Bac™ E. Coli using blue-white color selection, then verifying the presence of SMN-cDNA's insert in the recombinant bacmids by PCR amplification using the M13 forward (-40) and M13 reverse primers. As a result, the recombinant bacmid (**3**) is selected.

2.2. Using the pBlueBacHis2 A vector:

- Digesting the pBlueBacHis2 A vector with BamHI and XhoI followed by dephosphorylation with calf intestinal alkaline phosphatase.

- Digesting the vector (**2**) pFastBac™ HTb/SMN-cDNA with BamHI and XhoI and isolating the resulting fragment containing the cDNA coding sequences of SMN protein, SMN-cDNA.

- Ligating the SMN-cDNA fragment to the pBlueBacHis2 A vector and introducing the ligation product in INVαF' E. Coli strain.

- Screening for inserts using blue-white color selection, as a result of which the vector (**4**) pBlueBacHis2 A/SMN-cDNA is selected.

3. The procedure for the construction of expression plasmids using the pET-28a (+) transfer vector for the purpose of obtaining human SMN protein in bacteria, comprising:

- Digesting the pET-28a (+) vector with BamHI and XhoI followed by dephosphorylation with calf intestinal alkaline phosphatase.

- Digesting the vector (**2**) pFastBac™ HTb/SMN-cDNA with BamHI and XhoI and isolating the resulting fragment containing the cDNA coding sequences of SMN protein, SMN-cDNA.

- Ligating the SMN-cDNA fragment to the pET-28a (+) vector and introducing the ligation product in INVαF' E. Coli strain.

- Screening for inserts based on the presence of white colonies, as a result of which the vector (**5**) pET-28a (+)/SMN-cDNA is selected.

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